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A Diagnostic Test for the Recognition of Tuberculosis in Primates; a Preliminary Report.

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(Plates I & II).

A practical and simple test which might be applied to captive Primates for the early recognition of tuberculous infection is urgently needed (Schroeder, 1938). Tuberculosis was recognized as a disease to be coped with in the late 1890's in many zoological gardens. Recently the wholesale use of the Rhesus Macaque (*Macaca mulatta*) by laboratories has further shown the immediate need for a test applicable to large groups of newly imported monkeys. Penrose, White, Brown and Pearson, of the Penrose Laboratory of the Philadelphia Zoological Society, adapted to monkeys the test used in dairy cattle by the Bureau of Animal Industry (Fox, 1923). The test was used successfully at the Philadelphia Zoological Garden by these early workers. Their successful results were continued in the hands of White and Fox (White & Fox, 1909). Alterations and improvements in the test were made which depended upon a thermal response to an injection of K.O.T. The interesting discovery by early workers, indicating that Primates have a wider diurnal temperature variation than most other animals, made it imperative to record pre-injection temperatures so that an increase subsequent to the injection of tuberculin could be properly evaluated against the pre-injection graph (Simpson & Galbraith, 1906).

The Bureau of Animal Industry early recognized the necessity of altering its test, which was dependant on a regular systemic temperature rise following the subcutaneous administration of tuberculin. It was necessary to simplify it and make unnecessary the detailed taking and recording of temperatures on which depended the accuracy of the test. They adopted the intradermal test, at first in conjunction with the ophthalmic installation of tuberculin, later with a dual intradermal test (caudal fold and vaginal labia), and finally with a single caudal fold intradermal injection which has given best results because of its simplicity. The subcutaneous test is still admittedly accurate.

European investigators have done some work but nothing on a sufficiently large scale to be conclusive. The complete bibliography of Primate tuberculosis prepared by Dr. Margaret A. Kennard of Yale University School of Medicine gives no clew to a satisfactory test, in Europe or elsewhere, for laboratory animals.

A modification of the Bureau of Animal Industry's intradermal test in cattle has been evolved in the New York Zoological Park for use in monkeys.[1]

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Site of Injection.

A simple site for injection, easily available in all Primates and readily seen, was to be selected. For intradermal testing, the skin on the back proved most satisfactory, but required shaving and would necessitate handling the monkey to read the test (monkeys usually face an observer and the shaved area could not easily be seen without handling). The belly and thighs, by repeated trials, would not permit easy intradermal administration. The tuberculin usually broke through to the subcutaneous tissues and rapidly spread. The eyelid was a location easily seen, which could be readily approached, and has remained our choice of site for the injection.

The Tuberculin.

It seemed important to select a tuberculin which could be prepared in great concentration, so that when the injection was made the greatest dose of sensitizing agent per cell could be given (Sabin & Joyner, 1938). It is a well-known fact that monkeys do not react allergically to proteins as well as humans or domestic animals (Kopeloff & Kopeloff, 1938). In addition, a tuberculin was required which would be standard wherever used, and one which would not lose potency. We selected Purified Protein Derivative as prepared by Dr. Florence Seibert of the Henry Phipps Institute of the University of Pennsylvania because it best met these requirements[2] (Seibert, 1934).[3] It was necessary to use a tuberculin for the first test which would have sufficient concentration to bring down all reactors, regardless of severity of reaction. The final tuberculin used, which proved to be quite harmless to all non-tuberculous Primates, was a 1% solution of P.P.D. (0.1 cc. = 1 mgm. P.P.D.).[4] (Human type of organism is usually demonstrated).

The Test.

The animal to be tested is restrained by placing its arms behind it, legs extended. It is placed on its back, head extending over the edge of a bench or table, arms pulled down over the edge of the table at a right angle to the long axis of the body. The operator approaches from behind and grasps the head in the left hand. A 1 cc. tuberculin syringe and ⅜-inch 27-gauge needle is used. The syringe is held in the right hand, the needle inserted (bevel up) just under the skin in the upper lid of the right eye close to the margin. One-tenth cc. of the tuberculin is injected. This quantity will leave a lentil-size raised area. The operation is simple and not hazardous for attendant, operator or monkey. The specimen is placed in a cage and observed, without further restraint, after 16 hours. A reaction will usually begin to make its appearance at this time in a tuberculous monkey, and persist for 72 hours, depending upon the severity of the reaction. Primates too large for usual manual restraint can be anesthetized with Nembutal (29 mgm. per kilo, intraperitoneally).

Interpretation of the Test.

A positive reaction consists of edema of the lid with redness and, on rare occasions, a small area of necrosis. The extent of the reaction is immaterial. All reacting monkeys should either be destroyed or used for short-

[2] In a personal communication from Dr. Florence B. Seibert the following data on the stability of P.P.D. were offered: "We have found that a 1% P.P.D. solution kept in the incubator at 37.5° for one month loses no potency. Furthermore, a 1% P.P.D. solution kept in the icebox for nearly 5 years has lost very little potency."

[3] Some laboratories are using O.T. (Human) 0.1 cc. = 1.0 mgm. tuberculin (= 0.2% P.P.D.).

[4] No systemic reaction was demonstrated in non-tuberculous Orang-utans, Chimpanzees, Gorillas, Gibbons, Baboons, the Macaques, Mangabeys, the more delicate Douracoulis and the related New World monkeys.

time experiments. Those monkeys having active, advanced clinical disease will often succumb to this test dose of 1 mgm. P.P.D. In early or terminal infections the monkey may not react. In terminal infection, the tuberculin will either kill the specimen or bring about a profound systemic reaction, in which event it should be destroyed. Retesting after two months is important to detect infection in those specimens which did not have sufficiently advanced disease at the time of the first test to produce a reaction.

Discussion.

The test as presented leaves much room for further experimentation. It has proved to be quite satisfactory, however, and is being used routinely in some laboratories and zoological gardens. Recently the author, with one assistant, tested 15 Rhesus monkeys in a laboratory in 35 minutes. This period of time included catching the monkeys, injecting the tuberculin, and returning the animals to their cages. The following day the readings were made simply by observing the monkeys in their cages. The value of such a test can be determined only by extensive trial to accumulate experience, statistics on a large scale, so that correlation of tuberculin tests and autopsies can be made.

Probably 90% of Primate tuberculosis is acquired between time of capture and delivery to the final user of the specimens (Schroeder, 1937). Testing at origin, before shipment, will eliminate the ultimate planting of tuberculosis in all monkeys shipped in the same crate with a tuberculous specimen. When laboratories and zoological gardens demand tuberculosis-free monkeys from commercial distributors, tuberculosis in Primates will no longer be a problem.

Conclusions.

1. Monkeys will react locally to concentrated tuberculins.

2. The site of injection is the subcutaneous tissue at the margin of the upper eyelid.

3. The allergin used is 1/10 cc. of a 1% solution of Purified Protein Derivative tuberculin (0.1 cc. = 1 mgm. P.P.D.).

4. Reactors show swelling and redness of the lid, visually evident without removing the specimens from their cages, in 16 hours, and persisting for 72 hours.

Bibliography.

Fox, H.

1923. Diseases in Captive Wild Mammals and Birds. J. B. Lippincott, Philadelphia, p. 519.

Kopeloff, L. & Kopeloff, N.

1938. A Shock Reaction in the Monkey. *Journ. Soc. Amer. Bact.* Jan., 1938 (Abstract number).

Sabin, F. R. & Joyner, A. L.

1938. Tubercular Allergy Without Infection. *Journ. Exp. Med.* Vol. 68, p. 659.

Schroeder, C. R.

1938. Acquired Tuberculosis in the Primate in Laboratories and Zoological Collections. *Journ. Amer. Pub. Health Assn.* Vol. 28, p. 469.

Seibert, F. B., Arson, J. D., Reichel, John, Clark, L. T. & Long, E. R .

1934. Purified Protein Derivative, a standardized tuberculin for uniformity in diagnosis and epidemiology. *Amer. Rev. Tuberc.* Suppl. Vol. 30, No. 6, p. 713.

Simpson, S. & Galbraith, J. J.
1906. Observations on the Normal Temperature of the Monkey and Its Diurnal Variation and on the Effect of Changes in the Daily Routine on This Variation. *Trans. Royal Soc. Edinb.* Vol. 45, pp. 65-104.

White, C. Y. & Fox, H.
1909. Results of Tuberculin Tests in Monkeys at the Philadelphia Zoological Garden. *Arch. Int. Med.* Vol. 4, p. 517.

EXPLANATION OF THE PLATES.

Plate I.

Fig. 1. Manner of restraining monkeys weighing up to 10 kilos, for injection of tuberculin. Larger specimens should be anesthetized. When the operator touches the eyelid with the needle the monkey usually closes his eyes, which facilitates the proper administration of the tuberculin.

Fig. 2. The 27-gauge needle, attached to a 1 cc. Luer end tuberculin syringe, is inserted bevel up in the lid near its lower margin, and 0.1 cc. is administered.

Plate II.

Fig. 1. An average reaction consists of edema with or without apparent redness. The edema prevents the specimen from completely uncovering the pupil. Such a reaction can be recognized without restraining the specimen for close observation. Some reactions are more intense, with pronounced redness involving adjacent structures and edema which may close the eye.

Photographs by Hans Gehrung, N. Y. State Psychiatric Institute.